INFLAMMATORY BOWEL DISEASE

Measurement of vitamin D levels in inflammatory bowel disease patients reveals a subset of Crohn's disease patients with elevated 1,25-dihydroxyvitamin D and low bone mineral density

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Objectives: Many patients with Crohn's disease (CD) have low bone mineral density (BMD) that may not be solely attributable to glucocorticoid use. We hypothesised that low BMD in patients with CD is associated with elevated circulating levels of the active form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). We further hypothesised that this was secondary to increased synthesis of $1,25(OH)_2D$ by inflammatory cells in the intestine. The aim of this study was to examine the relationship between $1,25(OH)_2D$ levels and BMD in patients with CD.

Methods: An IRB approved retrospective review of medical records from patients with CD (n = 138) or ulcerative colitis (UC, n = 29). Measurements of vitamin D metabolites and immunoreactive parathyroid hormone (iPTH) were carried out. BMD results were available for 88 CD and 20 UC patients. Immunohistochemistry or real time reverse transcription-polymerase chain reaction (RT-PCR) for the enzyme 1α-hydroxylase was performed on colonic biopsies from patients with CD (14) or UC (12) and normal colons (4).

Results: Inappropriately high levels of serum $1,25(OH)_2D$ (>60 pg/ml) were observed in 42% of patients with CD compared with only 7% in UC, despite no differences in mean iPTH. Serum $1,25(OH)_2D$ levels were higher in CD (57 pg/ml) versus UC (41 pg/ml) (p = 0.0001). In patients with CD, there was a negative correlation between $1,25(OH)_2D$ levels and lumbar BMD ($r = -0.301$, p = 0.005) independent of therapeutic glucocorticoid use. $1,25(OH)_2D$ levels also correlated with CD activity. Lastly, immunohistochemistry and RT-PCR demonstrated increased expression of intestinal 1α-hydroxylase in patients with CD.

Conclusions: These data demonstrate that elevated $1,25(OH)_2D$ is more common in CD than previously appreciated and is independently associated with low bone mineral density. The source of the active vitamin D may be the inflamed intestine. Treatment of the underlying inflammation may improve metabolic bone disease in this subgroup of patients.

A serious and silent complication of inflammatory bowel disease (IBD) is the development of osteoporosis.[1–4] Estimates of osteopenia in IBD range from 31% to 59%[5 6] and osteoporosis from 5% to 41%.[1 7–10] Some studies have found that osteoporosis is more prevalent in patients with Crohn's disease (CD) than in those with ulcerative colitis (UC).[1 4 11–13] Other studies however, have found similar degrees of bone loss in CD and UC.[14 15] Lower bone mineral density (BMD) may be present at diagnosis,[13 16] suggesting factors other than medication may contribute to bone loss. The consequences of low BMD in patients with IBD include an increased risk of vertebral or hip fractures and their associated morbidity.[17–20] Indeed, recent data suggest that the risk of fractures in patients with CD may be underestimated. In a prospective study of CD patients, asymptomatic fractures were found in 14% of steroid free patients (including steroid naïve patients) and 15% of steroid dependent patients.[21] Similar results were reported by Klaus and colleagues.[22] These data suggest that the reasons for increased fracture and low BMD in patients with CD are complex.

Because of the prevalence of osteoporosis in patients with CD and the increased risk of fractures, it is important to identify risk factors resulting in bone loss. Approximately 40–70% of patients with CD are treated with glucocorticoids and

35% become dependent on this form of therapy.[23 24] Glucocorticoid use has generally been accepted as the principal cause of bone loss in these patients although some have not found an association.[10 14 25–31] However, in addition to glucocorticoids, other factors are proposed to contribute to accelerated bone loss in patients with CD. These factors include malabsorption of nutrients,[32 33] intestinal resections,[34] smoking,[35] inflammatory cytokines,[8 36] and vitamin D deficiency.[37–40] Pre-vitamin D generated in the skin by UVB radiation is converted in the liver to 25-hydroxyvitamin D (25(OH)D) and subsequently to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) in the renal tubular epithelium. The 1α-hydroxylase enzyme mediating the conversion to $1,25(OH)_2D$ is a rate limiting enzyme that is tightly regulated by parathyroid hormone (PTH) in response to serum calcium. Therefore, deficiency of 25(OH)D results in hypocalcaemia, increased PTH levels, and a rise in $1,25(OH)_2D$.[41–44] The principal action of $1,25(OH)_2D$ is mobilisation of skeletal

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Abbreviations: IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; BMD, bone mineral density; 25(OH)D, 25-hydroxyvitamin D; 1, $25(OH)_2D$, 1,25-dihydroxyvitamin D; PTH, parathyroid hormone; iPTH, immunoreactive parathyroid hormone; DXA, dual energy x ray absorptiometry; PCR, polymerase chain reaction; VDR, vitamin D receptor; mHBS, modified Harvey-Bradshaw

calcium stores through an increase in bone osteoclastic activity. Although vitamin D deficiency can be detrimental, so can inappropriately high levels of the hormonally active $1,25(OH)_2D$. When generation of $1,25(OH)_2D$ is not under the tight regulation of PTH, osteoclast resorption of bone may be accelerated resulting in decreased BMD. While calcium and vitamin D supplementation is recommended for the management of bone loss associated with IBD,[45–47] not all studies have identified vitamin D deficiency in these patients[25 34] or a benefit from vitamin D supplementation.[48]

The current study emanates from our observation that CD patients undergoing routine evaluation for metabolic bone disease were frequently found to have increased—not decreased—levels of the active hormonal form of vitamin D, $1,25(OH)_2D$. A review of patients' records found that almost half of the CD patients investigated had abnormally elevated levels of $1,25(OH)_2D$ in the absence of hyperparathyroidism. These data are reminiscent of case studies demonstrating increased $1,25(OH)_2D$ in CD[49] and sarcoidosis[50 51] associated with hypercalcaemia and granulomata. CD patients with elevated $1,25(OH)_2D$ in our cohort had lower BMD. Levels of $1,25(OH)_2D$ correlated with CD activity. Our study also demonstrated an increase in expression of the 1α-hydroxylase enzyme in the inflamed intestine of CD patients. These novel data suggest that elevated $1,25(OH)_2D$ is another risk factor in the development of low BMD in patients with CD and that the source of its production is the inflamed intestine.

METHODS
Patient analyses
The study was approved by the Cedars-Sinai Institutional Review Board and the Scientific Advisory Committee of the General Clinical Research Center for review of patients' medical records. During a nine month period from January 2002 to August 2002, the charts of 167 adult patients attending the Cedars-Sinai Inflammatory Bowel Disease Center were reviewed. As part of the evaluation for metabolic bone disease, patients routinely had evaluation of vitamin D metabolites and immunoreactive parathyroid hormone (iPTH) levels. Charts were examined for history of glucocorticoid use, which was categorised as none, low (<6 months of exposure and/or <10 mg/day average dose), and high (>6 months of exposure on at least prednisone 10 mg or equivalent) (prednisolone and prednisone are similar with respect to glucocorticoid and mineralocorticoid activity) and results of BMD by dual energy x ray absorptiometry (DXA). Modified Harvey-Bradshaw index was calculated from patients' charts by physicians using data from the clinic visit at the time of the vitamin D draw.[52] Patient clinical characteristics are shown in table 1. The group consisted of 138 patients with CD (mean age 37.7 (SEM 1.1) years) and 29 with UC (38.1 (3.3) years). Serum 25(OH)D and $1,25(OH)_2D$ values were determined by competitive protein binding assay (Esoterix (Endocrine Sciences), Woodland Hills, California, USA). Serum iPTH levels were determined by immunoradiometric assays (Nichols Institute, San Juan Capistrano, California, USA). Calcium, albumin, and creatinine were determined spectrophotometrically. Fractional urinary calcium excretion was measured in a fasting timed two hour collection of urine analysed for calcium and creatinine. Serum biochemistry results in patients with IBD were compared with a cohort of 96 normal subjects (mean age 40.0 (1.0) years) that were matched for age and sex from a database of hormonal values available at Nichols Institute.

BMD of the lumbar spine and non-dominant proximal femur was assessed in IBD patients by DXA on the same Lunar DPX machine (Lunar Corp., Madison, Wisconsin,

Table 1 Clinical characteristics of inflammatory bowel disease patients

	CD	UC
Sex (M/F)	75/63	17/12
Age at diagnosis (y)*	25.9 (1.3)	28.6 (3.8)
Age at study entry (y)*	37.7 (1.1)	38.1 (3.3)
Postmenopausal	9	3
Glucocorticoid use (none/low/high) (%)	15/18/67	5/28/67
Small bowel resection (Y/N)	35	0

UC, ulcerative colitis; CD, Crohn's disease.
*Values are mean (SEM).

USA); coefficients of variation for measurement of BMD in the spine and femoral neck were 1.0% and 1.2%, respectively. Areal BMD measurements were compared with a sex matched age matched normative population ("Z" score). Milder bone loss or osteopenia was defined as a BMD of 1.0–2.49 standard deviations below a control population at peak bone mass, and osteoporosis as BMD <2.5 standard deviations below a control population at peak bone mass.[53 54]

Immunohistochemistry
Immunohistochemical analysis of α-hydroxylase expression was carried out using an antiserum raised against mouse renal 1α-hydroxylase. The antiserum was synthesised using an antigenic region of the reported mouse amino acid sequence (peptide 266–289) which was 70% homologous to the reported human 1α-hydroxylase sequence. The 1α-hydroxylase fragment was synthesised as an eight branched multiantigenic peptide and used to immunise a single sheep. An IgG fraction was subsequently prepared from the immune serum (The Binding Site, Birmingham, UK). Preliminary studies using a human proximal tubule cell line (HKC-8) which expresses 1α-hydroxylase activity confirmed the specificity of the antiserum for the 56 kDa 1α-hydroxylase protein.[55] Western blot analysis identified a single 1α-hydroxylase protein species in these cells, and expression of this was downregulated in the presence of $1,25(OH)_2D$ and upregulated by PTH.[55] The antiserum was subsequently used to localise 1α-hydroxylase in both renal and extrarenal tissues.[56 57]

Real time polymerase chain reaction (PCR) analyses
Human colonic biopsies were obtained from patients undergoing colonoscopies who consented to having tissue donated for research purposes. This study was reviewed and approved for human subject participation by the Cedars-Sinai Institutional Review Board. Tissue used for this study included mucosal biopsies from patients having screening colonoscopies (n = 4) and patients with UC (n = 12) or CD (n = 14). Tissue was ground up in RNA Stat-60 with a motorised RNase Free Pellet Pestle (VWR, San Diego, California, USA). Total RNA was isolated using RNA Stat-60 according to the manufacturer's protocol. Quantitative real time PCR was conducted for vitamin D receptor (VDR) and 1α-hydroxylase using the following TaqMan probes and primers: VDR, forward primer 5'-CTT CAG GCG AAG CAT GAA GC-3', reverse primer 5'-CCT TCA TCA TGC CGA TGT CC-3', probe 5'-AAG GCA CTA TTC ACC TGC CCC TTC AA-3'; 1α-hydroxylase, forward primer 5'-CAC CCG ACA CGG AGA CCT T-3', reverse primer 5'-TCA ACA GCG TGG ACA CAA ACA-3', probe 5'-TCC GCG CTG TGG GCT CGG-3'. TaqMan probes and primers for the internal reference gene, β-actin, were as follows: forward primer 5'-CAT CCT CAC CCT GAA GTA CC-3', reverse primer 5'-GCT CAT TGT AGA AGG TGT GG-3', probe 5'-CAC GGC ATC GTC ACC AAC TG-3'. A total of 1 µg of RNA was reverse transcribed using Superscript III
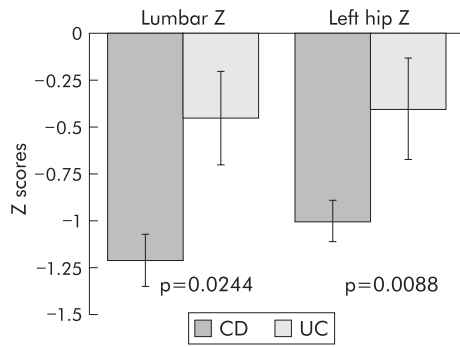
Figure 1 Crohn's disease (CD) patients have lower bone mineral density than ulcerative colitis (UC) patients. Bone mineral density, determined as age matched Z scores, was assessed in patients with CD or UC. Median scores are shown for the lumbar spine (n = 20 for UC; n = 96 for CD) and the left hip (n = 18 for UC; n = 94 for CD) with standard error bars. p values show statistically significant differences in CD values compared with equivalent UC analyses.
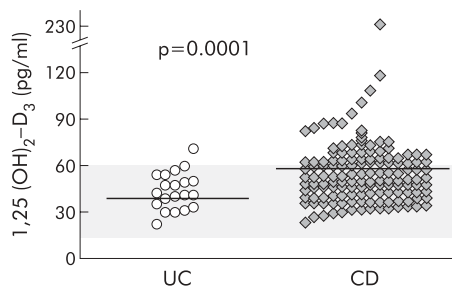

Figure 2 Circulating levels of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) are significantly higher in patients with Crohn's disease (CD) compared with ulcerative colitis (UC). The normal range of $1,25(OH)_2D$ is indicated by the shaded area (15–60 pg/ml) and mean $1,25(OH)_2D$ levels are indicated by the horizontal lines (57.77 pg/ml for CD and 41.3 pg/ml for UC; p = 0.0001). Overall, 42% of patients with CD had elevated $1,25(OH)_2D$ compared with only 7% of UC patients.

(Invitrogen). The following conditions were used: 50°C for two minutes, 95°C for two minutes, then 50 cycles at 95°C for 15 seconds and 60°C for one minute. Assays were performed following the predeveloped TaqMan assay reagents protocol (Applied Biosystems, Foster City, California, USA) in an iCycler (Bio-Rad, Hercules, California, USA). The iCycler

Optical System Interface (Bio-Rad) was used to analyse and quantitate samples.

Statistical analysis

Statistical analysis was performed using SAS computer software (version 6.10; SAS Institute, Inc., Cary, North Caroline, USA, 1994).[58] Quantitative variables are described as means (SEM) throughout. Non-parametric statistical tests were used to test differences in quantitative variables between the two groups. The Spearman correlation coefficient was used to examine the relationship between vitamin D levels and BMD. Two tailed Pearson correlation analysis was used to examine the correlation between high or low $1,25(OH)_2D$ and PTH. Multivariate analysis was performed to test the association between variables that were significantly associated with low BMD from the univariate analyses.

RESULTS

Patients with Crohn's disease have lower BMD than patients with ulcerative colitis

Multiple aetiologies have been suggested for the low BMD associated with CD. BMD results were available for 88 patients with CD and 20 patients with UC. Age matched Z scores were significantly lower in patients with CD compared with UC (fig 1). Both groups had comparable exposure to glucocorticoids (table 1). Although our small study found lower BMD in CD compared with UC, other population based studies have not found this difference.[14]

Circulating levels of $1,25(OH)_2D$ are elevated in patients with Crohn's disease

Some studies have reported low 25(OH)D in patients with CD[39 40] whereas a case report has described elevated circulating levels of $1,25(OH)_2D$.[49] We asked whether patients with CD had normal circulating levels of the active vitamin D hormone $1,25(OH)_2D$. Compared with patients with UC, mean serum concentration of $1,25(OH)_2D$ was significantly increased in adult patients with CD (table 2, fig 2). Serum $1,25(OH)_2D$ levels were elevated (>60 pg/ml) in 57 of 138 patients (42%) with CD but in only two of 29 patients (7%) with UC. Mean $1,25(OH)_2D$ concentration in the CD group was 57.8 (2.5) pg/ml and differed significantly from the mean value for UC patients (41.3 (2.8)) (p<0.0001). The increase in $1,25(OH)_2D$ could not be explained by serum levels of substrate 25(OH)D or iPTH, as no patient had low 25(OH)D with accompanying secondary hyperparathyroidism

Table 2 Circulating vitamin D metabolites and parathyroid hormone balance in adult patients with inflammatory bowel disease

	$1,25(OH)_2D$ (pg/ml)	25(OH)D (ng/ml)	iPTH (pg/ml)
Normal (n = 96)			
Mean (SEM)	42.1 (1.4)	27.0 (0.8)	32.3 (0.8)
Median	41.5	26	29
Range	15–85	5–57	11–74
CD (n = 138)			
Mean (SEM)	57.8 (2.5)	24.2 (1.2)	32.6 (2.4)
Median	54	25.5	28.5
Range	24–233	9–66	10.6–73
UC (n = 29)			
Mean (SEM)	41.3 (2.8)	25.9 (3.3)	28.8 (7.9)
Median	42	30	24.8
Range	18–72	7–48	10–72
t test (p values)			
Normal v CD	<0.0001	0.35	0.45
Normal v UC	0.31	0.06	0.19
CD v UC	0.0001	0.12	0.28

CD, Crohn's disease; UC, ulcerative colitis; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; iPTH, immunoreactive parathyroid hormone.

Table 3 Calcium to creatinine excretion in Crohn's disease (CD) patients is elevated compared with the normal population

Ca/creat	n	Mean	Range	SD	p Value
Controls	96	0.066	0.01–0.12	0.031	<0.001
CD	65	0.107	0.006–0.362	0.07	

(table 2). Indeed there was no correlation between iPTH and $1,25(OH)_2D$ ($r = 0.06$, $p = 0.6$). We also examined the relationship between iPTH and $1,25(OH)_2D$ in those patients with elevated $1,25(OH)_2D$ (>60 pg/ml) and again found no correlation ($r = 0.184$, $p = 0.28$). In the CD cohort, serum $1,25(OH)_2D$ concentration was not correlated with patient sex, duration of disease, cumulative glucocorticoid dose, or history of small bowel resection (data not shown). In our study, the positive predictive value for elevated $1,25(OH)_2D$ in identifying CD was 97% whereas the negative predictive value of normal $1,25(OH)_2D$ levels was 75%.

Elevated $1,25(OH)_2D$ levels may result in increased calcium absorption from the intestine, resulting in hypercalcaemia. None of our patients demonstrated hypercalcaemia (mean calcium level 9.3 (SD 0.49)) and there was no correlation between serum calcium and $1,25(OH)_2D$ levels. Serum phosphate levels were not different in control and IBD patients (data not shown). We hypothesised that elevations in serum calcium would be balanced by an increase in urinary calcium excretion. Calcium-creatinine clearance data were available for 65 CD patients. These data were compared with an age and sex matched control group of 96 patients. Our data demonstrated that calcium excretion was significantly higher in CD patients compared with controls (table 3).

We next addressed whether serum levels of $1,25(OH)_2D$ correlated with activity of the underlying CD. Modified Harvey-Bradshaw (mHBS) indices were calculated from patients' clinic visits at the time serum specimens were obtained for vitamin D analysis. In total, mHBS could be calculated for 72 patients in the cohort. There was a significant positive correlation between mHBS and $1,25(OH)_2D$ levels ($r = 0.266$, $p = 0.024$). When stratified by corticosteroid use at the time of vitamin D sampling, a significant correlation was found between mHBS and $1,25(OH)_2D$ levels in patients taking concurrent corticosteroids ($r = 0.68$, $p = 0.002$). This correlation did not exist in the absence of concurrent corticosteroid usage ($r = 0.106$, $p = 0.473$). Forty per cent of patients with high $1,25(OH)_2D$

levels were concurrently taking corticosteroids at the time of vitamin D sampling compared with 34% of patients with normal (<60 pg/ml) $1,25(OH)_2D$ levels ($p = 0.5$).

$1,25(OH)_2D$ levels are inversely correlated with BMD in Crohn's disease patients

Although circulating concentrations of vitamin D are important for normal calcium homeostasis and bone metabolism, high levels of the active hormone can result in increased bone resorption with a concomitant decline in BMD. We therefore examined the correlation between BMD (Z scores) and $1,25(OH)_2D$ levels in patients with CD. Our data demonstrated a significant negative correlation between BMD and $1,25(OH)_2D$ levels (table 4, fig 3). This correlation remained in patients in the high glucocorticoid use group (data not shown). Furthermore, multiple regression analysis for risk factors associated with low BMD demonstrated that glucocorticoid use and high $1,25(OH)_2D$ levels were independent risk factors for low BMD (table 5). These data suggest that, in addition to glucocorticoids, high levels of $1,25(OH)_2D$ levels are an important risk factor for the development of osteoporosis in patients with CD.

Intestinal expression of 1α-hydroxylase is increased in patients with Crohn's disease

The enzyme 1α-hydroxylase converts 25(OH)D to active hormonal $1,25(OH)_2D$ and is found predominantly in the kidney. However, other granulomatous diseases such as sarcoidosis are characterised by increased expression and activity of 1α-hydroxylase by activated macrophages.[50 59 60] We reasoned that lamina propria mononuclear cells in patients with CD might express 1α-hydroxylase activity. Immunohistochemical analysis of 1α-hydroxylase was carried out using colonic biopsies from patients with active and quiescent CD and compared with renal epithelial cells and a sarcoid granuloma as positive controls. Typical staining shown in fig 4 indicated that the enzyme was present in normal colon but with increased expression in inflamed biopsies from CD patients. Compared with colon from a representative patient with quiescent CD (fig 4C), colon from a patient with active CD, complicated by elevated serum $1,25(OH)_2D$ levels, showed intense submucosal staining for 1α-hydroxylase in epithelial cells (fig 4D). Increased expression of the enzyme was also demonstrated in macrophages and multinucleated giant cells (fig 4E) similar to that observed in sarcoidosis associated granulomas (fig 4F); the latter disease is the prototypical granuloma forming disease associated with vitamin D mediated hypercalciuria and
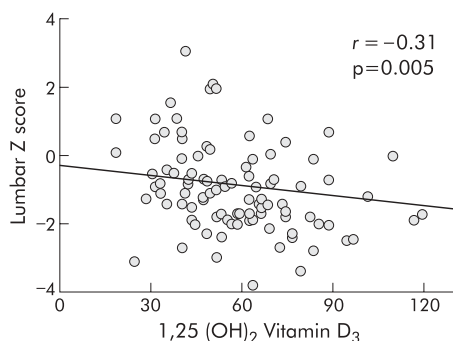

Figure 3 Circulating levels of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) are inversely correlated with bone mineral density in patients with Crohn's disease. The correlation between lumbar Z scores and $1,25(OH)_2D$ levels is shown. The r value indicates Spearman's correlation coefficient.

Table 4 Circulating levels of 1,25-dihydroxyvitamin D are inversely correlated with bone mineral density (BMD) in patients with Crohn's disease

BMD site	n	r	p Value
Left hip Z score	79	−0.227	0.044
Lumbar Z score	79	−0.310	0.005

Age matched BMD Z scores (lumbar and left hip) for Crohn's disease patients. Sample number (n), correlation coefficient (r), and statistical significance value (p) were determined.

Table 5 Multiple regression analysis for variables associated with lumbar Z scores in patients with Crohn's disease

Variable	r	p Value
1,25(OH)$_2$D	−0.011	0.0429
Small bowel resection	−0.049	0.8834
Years since diagnosis	−0.007	0.6432

1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; r, correlation coefficient.

hypercalcaemia.[8] In addition to immunohistochemistry, real time PCR of colonic biopsies from patients with CD or UC demonstrated that patients with CD expressed higher levels of 1α-hydroxylase whereas both UC and CD patients had reduced expression of VDR compared with normal colonic biopsies (fig 5A, 5B). We conclude from these data that lamina propria mononuclear cells are capable of expressing 1α-hydroxylase which, in the presence of the 25(OH)D substrate, may result in local synthesis of 1,25(OH)$_2$D.

DISCUSSION

Osteoporosis is a well recognised complication of CD and UC.[4] [14] [15] What is less clear however are the factors that result in its development and the best approach to its treatment and prevention.[17–19] [61–63] Based on our findings, we suggest that elevated 1,25(OH)$_2$D is an additional risk factor predisposing to development of osteoporosis in a subset of CD patients.

Our finding of normal 25(OH)D and elevated 1,25(OH)$_2$D is in contrast with previous studies demonstrating low 25(OH)D and secondary hyperparathyroidism in patients with CD.[39] [40] Some have suggested vitamin D supplementation in patients with CD to prevent bone loss[47] whereas others have found normal 25(OH)D and serum calcium levels in CD.[64] The reasons for the divergent results may be geographic location (Southern California versus northern climates) and limited intestinal resections in recently ascertained patient cohorts. Patients with extensive surgical resections for CD were commonplace at the time most of the previous vitamin D studies were performed[33] [37] and may have had reduced absorption of cholecalciferol and 25(OH)D.[65] The change in treatment of CD away from surgery and, to some extent, away from systemic glucocorticoids, requires a revisiting of established tenets with respect to vitamin D.

Our study demonstrated an inverse correlation between 1,25(OH)$_2$D levels and BMD. Although we demonstrated this correlation, it is not possible to demonstrate causality between the two. We believe that the increase in systemic 1,25(OH)$_2$D is the result of the underlying inflammation in CD. Consistent with this notion, disease activity correlated with 1,25(OH)$_2$D levels, and biopsies from patients with active CD and elevated 1,25(OH)$_2$D demonstrated increased expression of 1α-hydroxylase required for conversion of 25(OH)D to its active 1,25(OH)$_2$D form. Elevated 1,25(OH)$_2$D may therefore be a direct cause of bone loss or a surrogate marker for the type of intestinal inflammation resulting in osteoporosis. Because of the retrospective nature of our study, it is not possible to determine whether the increase in 1,25(OH)$_2$D predates the onset of osteoporosis or is related to the severity of the underlying CD. Multivariate analyses demonstrated that 1,25(OH)$_2$D is an independent risk factor for low BMD but the use of corticosteroids was high in this patient population. Nevertheless, our findings help to identify a subgroup of patients with an additional risk factor for osteoporosis and one that can be used to make decisions on further evaluation and therapy.

We have shown previously that intestinal epithelial cells normally express 1α-hydroxylase.[56] The present study shows for the first time that lamina propria mononuclear cells also
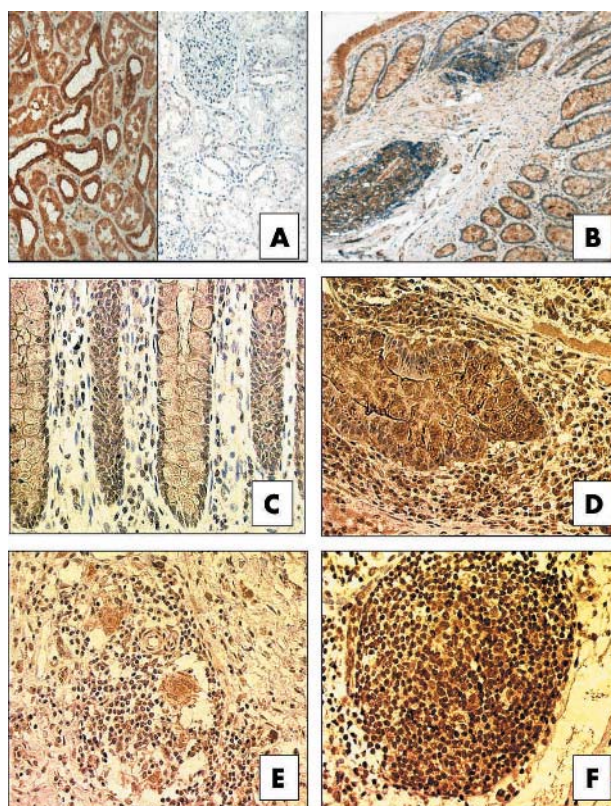

Figure 4 Expression of 1α-hydroxylase in normal colon and Crohn's disease. (A) Positive control demonstrating characteristic anti-1α-hydroxylase staining in human renal tubular epithelial cells (left, 250× magnification). Non-specificity control of human kidney stained with antiserum preincubated with immunising peptide (right, 250× magnification). (B–D) Immunostaining for 1α-hydroxylase in colon from a normal subject (B) and subjects with quiescent (C) and active Crohn's disease (D), respectively (350× magnification). (E, F) Comparison of immunostaining of 1α-hydroxylase in granulomas from the colon of a patient with Crohn's disease and elevated serum 1,25-dihydroxyvitamin D (E) and the skin of a patient with active sarcoidosis (F) (350× magnification).

express 1α-hydroxylase and therefore have the ability to generate 1,25(OH)$_2$D locally. Furthermore, in the presence of intestinal inflammation, the increased number of lamina propria mononuclear cells combined with the availability of 25(OH)D as the substrate for 1α-hydroxylase results in the generation of higher levels of 1,25(OH)$_2$D. We propose a model in which excess 1,25(OH)$_2$D from the inflamed gut spills over into the circulation and may have the unintended effect of contributing to metabolic bone disease in patients with CD (fig 6). This situation is similar to that seen in other granulomatous diseases such as sarcoidosis[51] in which mature macrophages express 1α-hydroxylase resulting in hypercalcaemia and hypercalciuria.[50] [59] [60] In both sarcoidosis and CD, the most likely function of extrarenal 1,25(OH)$_2$D production is as an endogenous feedback response to inflammation. A variety of studies have documented the immunosuppressive properties of 1,25(OH)$_2$D, stimulating interest in the potential use of synthetic analogues of vitamin D as therapy for autoimmune disease and transplantation rejection.[66–68] However, recent data from our group suggest that vitamin D may also fulfil a protective role, with macrophages, dendritic cells, and epithelial cells being potential sources of immunomodulatory 1,25(OH)$_2$D.[69] [70]. CD is characterised by Th1 mediated intestinal inflammation with a subset of patients demonstrating microgranuloma.[71] [72] Thus the goal of local 1,25(OH)$_2$D production may be

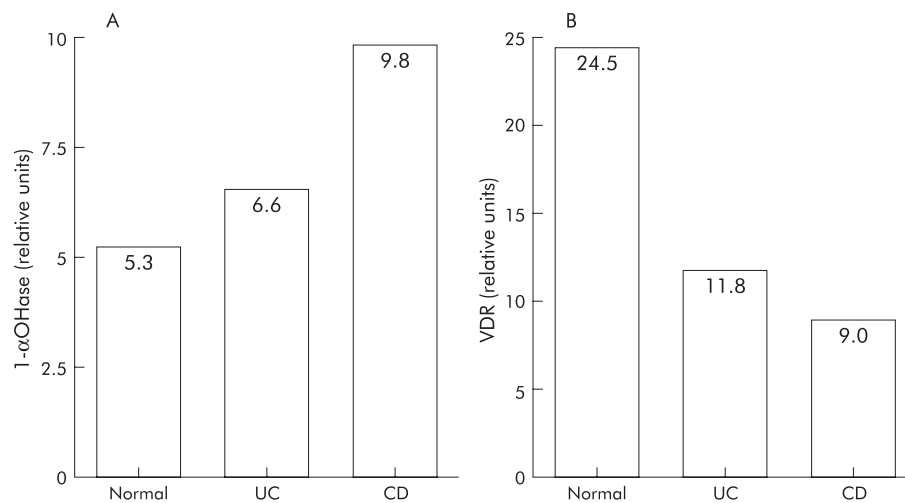**Figure 5** Expression of 1α-hydroxylase and vitamin D receptor (VDR) in normal, Crohn's disease (CD), and ulcerative colitis (UC) colons. 1α-Hydroxylase (A) and VDR (B) mRNA expression was assessed by quantitative real time polymerase chain reaction. These data represent an average of 10 mucosal biopsy samples each from patients with CD or UC.

suppression of Th1 cytokine secretion and T cell proliferation, as we and others have described in other systems.[44 73 74] Indeed, in animal models of colitis limiting the substrate for $1,25(OH)_2D$ or using mice with disruption of the VDR gene results in worse colitis.[75 76] Clearly, in many instances of CD, local synthesis of $1,25(OH)_2D$ may not be sufficient to suppress tissue inflammation, and in these cases unregulated 1α-hydroxylase may lead to raised circulating levels of the hormone. As with sarcoidosis, the extent to which this occurs is likely to be dependent on the severity of the disease but also on serum levels of 25(OH)D or, in other words, vitamin D status.

The clinical implications of our work are several. Firstly, elevated $1,25(OH)_2D$ may serve as a marker of CD. The positive and negative predictive values for supranormal

(>60 pg/ml) $1,25(OH)_2D$ in patients with CD are 97% and 75%, respectively. These numbers are comparable with serological tests currently available for distinguishing CD from UC.[77–79] Secondly, clinicians frequently recommend that patients take calcium and vitamin D supplementation to prevent or treat osteopenia and osteoporosis in patients with IBD. Based on our work, measuring vitamin D metabolites is important in identifying those patients with an additional risk factor for developing low BMD. Moreover, we have also found that patients with elevated $1,25(OH)_2D$ frequently have hypercalciuria and may be prone to renal nephrolithiasis (data not shown). The correct intervention in these patients is not established. Strategies to reduce bone loss in these patients may include non-glucocorticoid treatment of the underlying IBD and potentially hydroxychloroquine which
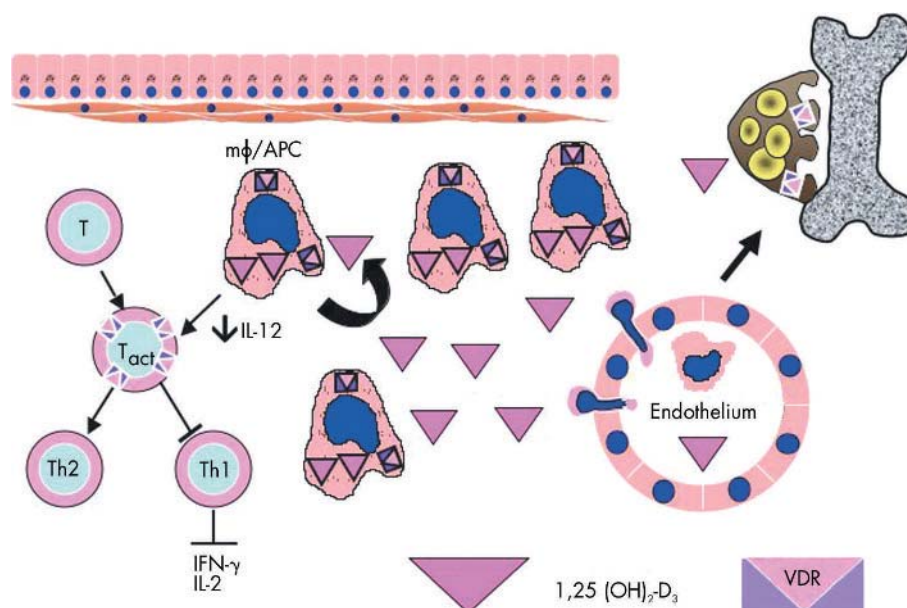

Figure 6 Model of elevated 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) in patients with Crohn's disease. The inflamed intestine in Crohn's disease is characterised by an infiltrate of mononuclear cells, including antigen presenting cells (APC) and macrophages (mΦ). These cells express the 1α-hydroxylase enzyme resulting in conversion of 25-hydroxyvitamin D to $1,25(OH)_2D$. T cells and APC/mΦ express vitamin D receptors (VDR). Thus $1,25(OH)_2D$ may have both paracrine and autocrine effects to suppress Th1-type differentiation. Excess local production of $1,25(OH)_2D$ may lead to spillage of the hormone into the systemic circulation and distant osteoclast activation and bone resorption. IFN-γ, interferon γ; IL-2, interleukin 2.

inhibits the conversion of 25(OH)D to 1,25(OH)$_2$D.[80] In sarcoidosis patients with hypercalcaemia secondary to elevated 1,25(OH)$_2$D, treatment with hydroxychloroquine is effective in reducing serum calcium.[81] Preliminarily, we have treated several CD patients with low dose (200 mg daily) oral hydroxychloroquine and lowered serum 1,25(OH)$_2$D levels and urinary calcium excretion (unpublished studies). Finally, if elevated 1,25(OH)$_2$D is an indirect marker of Th1 mediated inflammation, we may be able to use this test to better predict who will respond to medical therapy directed at Th1 cytokines in CD. More research will need to be done to better characterise this interesting subgroup of patients.

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